

LOCALIZATION OF INSULINOMAS AND ISLET CELL HYPERPLASIAS BY PANCREATIC
VEIN CATHETERIZATION AND INSULIN ASSAY.

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Abstract

In local anesthesia percutaneous transhepatic portal vein catheterization and transfemoral portal and caval catheterizations were performed in five patients with symptoms of organic hypoglycemia. During the investigation pancreatic phlebography revealed the pancreatic venous anatomy. Blood obtained from the coeliac artery, caval branches and pancreatic veins was assayed for insulin employing two different radioimmunoassays. Pathologically high, pancreatic arteriovenous insulin differences were seen with both radioimmunoassay techniques in two insulinoma patients (Pats. Nos. I and V) and in two patients with islet cell hyperplasia (Pats. Nos. III and IV). In one of the insulinoma patients (Pat. No. II) one of the assays failed to detect the tumor insulin. This inconsistency still remains unexplained. Angiography revealed the presence of a pancreatic abnormality only in patients Nos. I and II. During operation two of the tumors were found by palpation and inspection (Pats. Nos. I and V). Pancreatic resections were performed according to the findings of pathological hormone differences in all five patients. Immunocytochemistry revealed that three of the patients suffered from insulin producing tumors (Pats. Nos. I, II and V) and two from local islet cell hyperplasia (Pats. Nos. III and IV). Catheterizations performed two months postoperatively confirmed radicality of the operation in all patients with the possible exception of patient No. II. In patient No. III recurrence was detected by catheterization ten months postoperatively.

Introduction

Hypoglycemia may be caused by pancreatic as well as extrapancreatic factors (18, 22, 23, 24, 29). Frequently, clinical symptoms as well as laboratory tests indicate the presence of an insulin producing tumor. Attempts to localize such tumors have been made by scintiscan (3), ultrasound (9) or arteriography. The method currently considered best - arteriography - has an accuracy of 40-60 per cent (5, 6, 20, 22, 23, 26, 27) and a false positive rate of about 5 per cent. Some tumors not detected preoperatively may be found by careful peroperative inspection and palpation. However, this procedure sometimes fails. At present, no specific surgical approach can be recommended for such tumors (19, 22, 23, 24, 25, 26, 27). To overcome this difficulty we have used pancreatic vein catheterization (7, 12) with blood sampling for hormone assay as a diagnostic and localization procedure for one insulinoma (11) and one islet cell hyperplasia (13). In this report we present results of pre- and postoperative pancreatic vein catheterization performed in three patients with insulinproducing tumors and in two patients with islet cell hyperplasia. Blood samples withdrawn during the catheterization were assayed for insulin with the use of two different antisera. Further the contribution of proinsulin to the total insulin immunoreactivity was determined.

Methods

Catheterizations were performed after 8 hours of fasting. Patients Nos. I and III were given glucose infusions (1.5 mg/kg bw/min) in order to avoid hypoglycemia during the investigations.

In local anesthesia percutaneous, transhepatic portal vein catheterization, percutaneous catheterization of the aorta and of the inferior vena cava were performed via the femoral artery and vein, respectively. Blood samples were withdrawn from different aortal and caval branches for hormone assay in order to exclude extrapancreatic tumors. The aortal catheter was then placed in the coeliac artery. After taken blood samples from different hepatic veins the caval catheter was placed in the right hepatic vein. Blood samples were withdrawn from different portal vein branches including the pancreatic veins. Fluoroscopy and phlebography revealed the exact position of the cathetertip during blood sampling. Blood samples were collected in ice chilled tubes, centrifuged and then serum was stored at -20°C until assayed. Sera were assayed for insulin either by the Wick separation technique or according to Heding. A guinea-pig anti-porcine insulin antiserum (raised against monocomponent porcine insulin) was used in the Wick technique (14, 30). In the Heding method (8) an anti-porcine insulin guineapig antiserum, serum M29, was used (kindly donated by Dr. Heding, Novo Research Laboratories, Copenhagen, Denmark). Serum M29 was drawn from a guineapig repeatedly immunized with porcine zink-protamine insulin emulsified in Freund's adjuvant.

Blood-glucose determinations were performed on coeliac artery samples with a glucose oxidase method.

Proinsulin concentrations were determined with the Wick separation technique after separation of sera (fig. 2 a, samples nos. 22,23,24) by gel-chromatography on a Sephadex G-50 superfine column (1.6 x 100 cm) as described elsewhere (15).

Tissue specimens were obtained at surgery, frozen in melting Freon-22, freeze-dried, vapor-fixed with formaldehyde (2) or diethylpyrocarbonate (DEPC) (17) and embedded in paraffin in vacuo. Sections cut at $3\ \mu$ were deparaffinized^x and allowed to react with antisera against insulin, glucagon^x, gastrin⁺ and human pancreatic polypeptide (HPP)⁺⁺. The site of antigen-antibody reaction was revealed either by the PAP-method of Sternberger (28) or with fluorescein-isothiocyanate (FITC)-labeled sheep anti-guineapig IgG or FITC-labeled sheep anti-rabbit IgG (SBL, Stockholm, Sweden). For demonstrating insulin and HPP, sections from formaldehyde-fixed specimens were routinely re-fixed for two hours in Bouin's fluid.

^xKindly donated by Dr. J.J. Holst, Department of Clinical Chemistry, Bispebjerg Hospital, Copenhagen, Denmark.

⁺Kindly donated by Prof. J.F. Rehfeld, Institute of Medical Biochemistry, University of Aarhus, Aarhus, Denmark.

⁺⁺Kindly donated by Dr. R.E. Chance, Lilly Research Laboratories, Indianapolis, Indiana, USA.

	Pat. no. I (E.J.)	Pat. no. II (A.O.)	Pat. no. III (U.H.)	Pat. no. IV (L.S.)	Pat. no. V. (I.C.)
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P R E O P E R A T I V E F I N D I N G S.

Palpation	Normal	Normal	Normal	Normal	Normal
Ultrasound	Not performed	Normal	Normal	Normal	Normal
Scintiscan	Not performed	Normal	Normal	Normal	Normal
Selective arteriography (coeliac artery):	A pancreatic cyst (5 cm diam.) dis- locating the pancre- atic tail upwards. Tumor in the tail of the pancreas.	A hypervascular raised area (10 mm diam.) in the left part of the pancreatic body.	A slight arterial dislocation in the pancreatic head.	Normal	Normal
Portal flebography	Normal	Normal	Normal	Normal	Normal
Pancreatic flebography	A slight disloca- tion of the v. ASPD.	Normal	Normal	Normal	Normal
Tripple catheteri- zation with blood sample for hormone assay	Insulin hyper- secretion from the left part of the pancreatic body (fig. 1 a).	Insulin hyper- secretion from the left part of the pancreatic body (fig. 2 a).	Insulin hyper- secretion from the tail of the pancreas (fig. 3 a).	Insulin hyper- secretion from the tail of the pancreas (fig. 4 a).	Insulin hyper- secretion from the tail of the pancreas (fig. 5 a)

P E R O P E R A T I V E F I N D I N G S.

Inspection	Pancreatic cyst and two tumors in the tail of the pancreas.	Normal	Normal	Normal	Tumor in the pancreatic tail.
Palpation	Pancreatic cyst and two tumors in the tail of the pancreas.	Normal	Normal	Normal	Tumor in the pancreatic tail.
Fine needle biopsy	Endocrine tumors	Endocrine tumor	Normal	Normal	Endocrine tumor
Histology and immunohistochemistry	Insulinomas	Insulinoma	Islet hyperplasia	Islet hyperplasia	Insulinoma

Table I

Pre- and peroperative findings in patients nos. I - V.

	IRI (μ U/ml)		Glucose (mmol/l)
1. v. lienal.	= 108 / 112	v. hep. = 147 / 91	2,4
2. v. lienal.	= 146 / 95	a. coel. = 200 / 113	
3. v. lienal.	= 865 / 704	v. hep. = 111 / 97	
4. v. portae	= 260 / 211	a. coel. = 115 / 106	
5. v. gastropipl. dx.	= 120 / 91	v. hep. = 160 / 93	5,4
u. v. mes. sup.	= 109 / 108	a. coel. = 111 / 99	
7. v. ASPD	= 155 / 304	v. hep. = 158 / 88	
8. GCT	= 93 / 101	a. coel. = 146 / 83	
9. v. mes. inf.	= 134 / 157	v. hep. = 105 / 101	6,5
10. v. port.	= 139 / 327	a. coel. = 150 / 140	
		v. hep. = 137 / 115	
		a. coel. = 160 / 259	
		v. hep. = 165 / 132	
		a. coel. = 143 / 129	
		v. hep. = 115 / 81	
		a. coel. = 110 / 156	
		v. hep. = 165 / 167	
		a. coel. = 90 / 179	
		v. hep. = 165 / 174	
		a. coel. = 76 / 163	

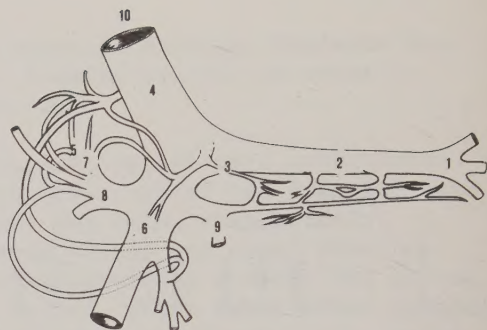


Fig. 1 a

Results of insulin determination with two immunoassay techniques (Wick/Heding) in patient No. I before operation.

	IRI (μ U/ml)		Glucose (mmol/l)
1. v. ASPD	= 146 / 200	v. hep. = 12 / 7	4,8
2. v. mes. sup.	= 5 / 1	a. coel. = 6 / 4	
3. v. lienal.	= 14 / 5	v. hep. = 9 / 3	4,7
4. v. portae	= 30 / 0	a. coel. = 5 / 0	
5. v. PSPD	= 50 / 0	v. hep. = 13 / 6	4,0
6. v. portae	= 26 / 9	a. coel. = 7 / 7	
7. v. DP	= 134 / 99	v. hep. = 13 / 0	
8. v. mes. inf.	= missed	a. coel. = 4 / 3	
9. v. jejunal	= 7 / 0	v. hep. = 15 / 1	
10. GCT	= missed	a. coel. = 7 / 0	
11. v. mes. sup.	= 9 / 0	v. hep. = 10 / 0	
		a. coel. = 3 / 0	
		v. hep. = 15 / 1	
		a. coel. = 13 / 0	
		v. hep. = 11 / 3	
		a. coel. = 9 / 1	
		v. hep. = 14 / 0	
		a. coel. = 6 / 1	
		v. hep. = 11 / 2	
		a. coel. = 7 / 1	

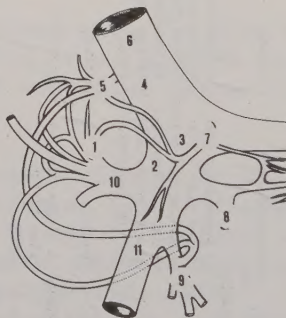


Fig. 1 b

Results of insulin determination with two immunoassay techniques (Wick/Heding) in patient No. I two months after operation.

	IRI (uU/ml)		Glucose (mmol/l)
1. v. mes. sup.	= 2	v. hep. = 6 a. coel. = 3	6,3
2. v. mes. sup. dist.	= 2		
3. v. mes. sup. (above 1)	= 3		
4. v. mes. inf.	= 3		
5. v. ASPD	= missed		
6. GCT	= 4		
7. GCT	= 5		
8. v. coronar.	= 4	v. hep. = 9 a. coel. = 5	8,2
9. v. port. centr.	= 64		
10. v. port. centr.	= 41	v. hep. = 10 a. coel. = 6	
11. v. DP	= missed	v. hep. = 15 a. coel. = 8	
12. v. DP	= 140		
13. v. DP	= 160		
14. v. DP	= 42		
15. v. DP	= 86		
16. v. port. confl.	= 16	v. hep. = 12 a. coel. = 8	7,3
17. v. port. centr.	= 36		

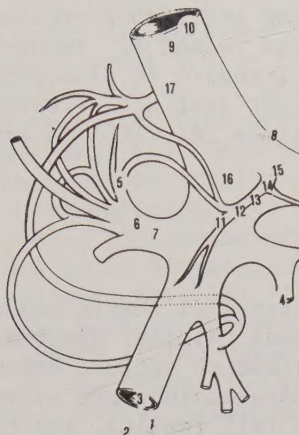
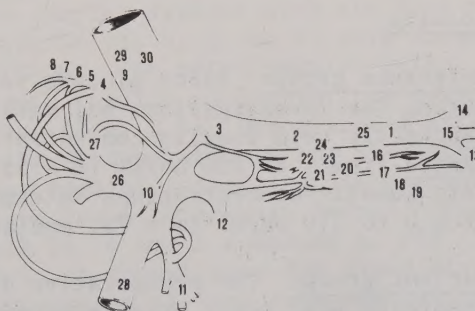


Fig. 1 c

Results of insulin determination with the Wick technique in patient No. I twelve months after operation.

	IRI (uU/ml)		Glucose (mmol/l)
1. v. lienal.	= missed	v. hep. = 13 / 25 a. coel. = 12 / 25	5,6
2. v. lienal.	= 119 / 304		
3. v. lienal.	= 9 / 23	v. hep. = 12 / 27 a. coel. = 8 / 21	
4. v. pancreat.	= missed		
5. v. pancreat.	= 5 / 19		
6. v. pancreat.	= 6 / 22	v. hep. = 11 / 24 a. coel. = 6 / 17	
7. v. pancreat.	= 4 / 15		
8. v. pancreat.	= 5 / 20		
9. v. portae	= 22 / 58	v. hep. = 9 / 28 a. coel. = -	
10. v. mes. sup.	= 4 / 13	v. hep. = 7 / 35 a. coel. = 5 / 26	
11. v. jejunal.	= 3 / 23		
12. v. mes. inf.	= 4 / 25		
13. v. lienal.	= 3 / 24	v. hep. = 6 / 42 a. coel. = 4 / 28	4,8
14. v. lienal.	= 3 / 20		
15. v. lienal.	= 2 / 30		
16. v. pancreat.	= 3 / 25		
17. v. pancreat.	= 6 / 70	v. hep. = 6 / 42 a. coel. = 4 / 21	
18. v. pancreat.	= 6 / 22		
19. v. pancreat.	= 5 / 21		



20. v. pancreat.	= 36 / 75	v. hep. = 5 / 26 a. coel. = 2 / 24
21. v. pancreat.	= 14 / 75	
22. v. pancreat.	= 35 / 452	v. hep. = 4 / 38 a. coel. = 2 / 24
23. v. pancreat.	= 17 / 104	
24. v. pancreat.	= 37 / 600	
25. v. lienal.	= - / 25	
26. GCT	= 7 / 22	
27. v. ASPD	= 5 / 21	
28. v. mes. sup.	= 5 / 18	
29. v. portae	= 18 / 43	
30. v. portae	= 11 / 40	

4,9

Results of insulin determination with two immunoassay techniques (Wick/Heding) in patient No. II before operation.

Fig. 2 a

This re-fixation procedure was not employed in case of DEPC-fixed specimens. Specificity controls were those recommended by Sternberger (28) and included the application of antigen-inactivated antisera. For details concerning the immunocytochemical procedure the reader is referred to references 16 and 17. Immunofluorescence preparations were analyzed in a Zeiss standard 18 fluorescence microscope equipped for epi-illumination with filter settings selected to give peak excitation at 490 nm (excitation maximum for FITC). A Xenon XBO 75 lamp served as light source.

Material

Five patients (1 female and 4 males) 31-70 years old, of normal weight, and without clinical symptoms of hypoglycemia or diabetes mellitus were catheterized to exclude abdominal malignancies. The insulin concentrations measured in these patients were used as reference values. Five patients (all females, and referred to as patients Nos. 1-V) 46-60 years of age with symptoms indicating hyperinsulinemia were catheterized preoperatively as well as two months postoperatively. Patient No. I was also catheterized 12 months after operation for follow up. Patient No. III was re-investigated 10 months after the operation because of suspected recurrence with hypoglycemic attacks.

Results

Reference group: Blood glucose values ranged from 4.3 to 6.5 mmol/l during the investigations and varied with maximally ± 0.8 mmol/l. Pancreatic vein insulin concentrations assayed with the Wick technique ranged from 5 to 200 uU/ml ($\bar{m} = 83.5$, median = 97, N = 18). Pancreatic vein insulin concentrations determined with the Heding technique ranged from 0 to 210 uU/ml ($\bar{m} = 72$, median = 55, N = 18).

Patient group: The preoperative diagnosis was based on clinical symptoms (patients Nos. I-V), Whipple's triad (patients Nos. I-V) and pathological glucose:insulin ratios during 10-36 hours of fasting (pats. Nos. I, II and V). In pats. Nos. III and IV intermittent pathological glucose:insulin ratios were found during fasting. The results of scintiscan, ultrasound, arteriography, portal flebography, pancreatic phlebography, pancreatic vein catheterization and preoperative findings are presented in Table I.

Blood glucose concentrations during the catheterizations are shown in Figures 1-5.

Serum analyses: In patient No. I (Fig. 1 a, sample No. 3), III (Fig. 3 a, sample No. 10-13, Fig. 6 a and Fig. 3 c, sample No. 4, 5), IV (Fig. 4 a, sample No. 4, 5) and V (Fig. 5, sample No. 13, 14, 16, 17) insulin concentrations as measured by both assays were much elevated over a well-defined

pancreatic portion. In patient No. II normal levels of insulin were found with the Wick radioimmunoassay (Fig. 2 a). High insulin concentrations in veins draining the area between the body and the tail of the pancreas were measured with the Heding method (Fig. 2 a, sample No. 22, 24). At explorative laparotomies a 3 x 4 cm large tumor was detected by inspection and palpation in patient No. I (11) and a tumor 0.8 cm in diameter was detected by inspection and palpation in patient No. V. Both tumors were peroperatively diagnosed as endocrine tumors by fine needle aspiration biopsy. The tumors were drained by the veins that were found to contain high insulin concentrations with the catheterization procedure. In patients Nos. II, III and IV no tumors were detected with inspection or palpation. During visualization of the veins containing high insulin concentrations in patient No. II a tumor 0.4 cm in diameter was found embedded in normal pancreatic tissue. In patients Nos. III (13) and IV the pancreas appeared normal and hence distal hemipancreatectomies were performed since, in both patients, the source of the raised insulin concentrations emanated from this portion of the organ. The postoperative courses were uneventful and the hypoglycemic attacks disappeared in all five patients. Preoperative pathological laboratory tests were postoperatively normalized. Two months after operation re-catheterization of patients Nos. I, III and IV revealed normal insulin concentrations as shown with both assay techniques (Fig. 1 b, 3 b). Samples withdrawn postoperatively from patient No. IV were assayed with the Wick method only (Fig. 4 b). Twelve months after operation re-catheterization of patient No. I revealed normal insulin values (Fig. 1 c). In patient No. II the insulin concentrations were found within the range of the reference group in the pancreatic venous blood samples assayed with the Wick method. However, five samples (Fig. 26, sample No. 1, 2, 3, 4, 6) were close to the upper limit of the reference group. The serum samples were also assayed with the Heding technique. Insulin concentrations were found above the range of the reference group (Fig. 2 b). Hypoglycemic attacks recurred in patient No. III 4-5 months postoperatively and re-catheterization 10 months after the operation revealed raised insulin concentrations in a vein which earlier had been found to contain normal concentrations of insulin (Fig. 3 c, sample No. 4 and 5 compared to Fig. 3 b, sample No. 2 and compared to Fig. 6 b, c) though very low blood glucose levels as seen from Fig. 3 c. Chromatography of sera from samples containing high insulin immunoreactivity as measured with the Heding but not with the Wick method (patient No. II, sample Nos. 22, 24) showed that all immunoreactive material migrated in the position of highly purified human insulin.

Proinsulin was found to make up less than one fourth of the total insulin immunoreactivity in all sera tested (see Table II).

Immunocytochemistry: In patients Nos. I and V the tumors were composed of intensely immunoreactive insulin cells which in patient No. V infiltrated the surrounding connective tissue capsule. The tumors were devoid of glucagon, gastrin or HPP-cells.

	IRI (μ U/ml)		Glucose (mmol/l)
1. v. portae	= 179 / 328	v. hep. = 13 / 36 a. coel. = 10 / 29	6,5
2. v. pancreat.	= 200 / 792	v. hep. = 17 / 32 a. coel. = 8 / 22	
3. v. pancreat.	= 150 / 680		
4. v. pancreat.	= 200 / 708		
5. v. pancreat.	= 73 / 252		
6. v. pancreat.	= 100 / 328		
7. v. pancreat.	= 200 / 752		
8. v. mes. sup.	= 8 / 22	v. hep. = 6 / 25 a. coel. = 4 / 17	6,4
9. v. mes. sup.	= 30 / 64		
10. v. PSPD	= 32 / 45		
11. v. PSPD	= 19 / 93		
12. v. portae	= 14 / 54		
13. v. portae	= 9 / 39		6,6

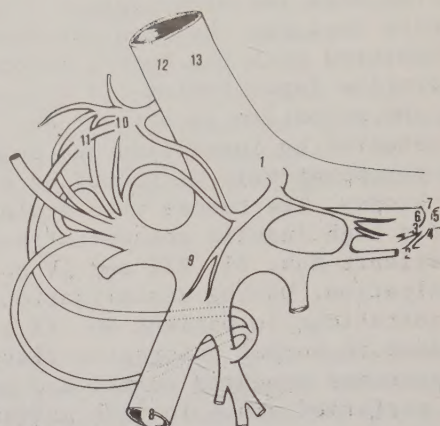


Fig. 2 b

Results of insulin determination with two immunoassay techniques (Wick/Heding) in patient No. II two months after operation.

	IRI (μ U/ml)		Glucose (mmol/l)
1. v. lienal.	= 13 / 0	v. hep. = 23 / 0 a. coel. = 11 / 0	
2. v. lienal.	= missed	v. hep. = 16 / 0 a. coel. = 11 / 0	
3. v. lienal.	= 45 / 12	v. hep. = 13 / 0 a. coel. = 11 / 0	
4. v. port.	= 56 / 16	v. hep. = 19 / 3 a. coel. = 14 / 0	9,5
5. v. mes. sup.	= 11 / 0	v. hep. = 36 / 3 a. coel. = 21 / 0	
6. v. mes. sup.	= 63 / 37	v. hep. = 19 / 1 a. coel. = 13 / 0	
7. v. PSPD	= 13 / 0	v. hep. = 22 / 0 a. coel. = 14 / 0	9,0
8. v. PSPD	= 71 / 47	v. hep. = 15 / 0 a. coel. = 17 / 0	9,5
9. v. PSPD	= 90 / 84	v. hep. = 28 / 3 a. coel. = 16 / 1	9,2
10. v. lienal.	= 165 / 304	v. hep. = 18 / 18 a. coel. = 15 / 0	
11. v. pancreat.	= 950 / 832	v. hep. = 29 / 9 a. coel. = 17 / 1	
12. v. pancreat.	= 1130 / 632	v. hep. = 19 / 4 a. coel. = 17 / 1	9,2
13. v. pancreat.	= 280 / 311	v. hep. = 20 / 3 a. coel. = 14 / 0	
14. v. ASPD	= 14 / 0	v. hep. = 21 / 1 a. coel. = 16 / 0	9,0
15. v. port.	= 58 / 36		
16. v. port.	= 62 / 53	v. hep. = 41 / 20 a. coel. = 19 / 0	
17. v. port.	= 59 / 39	v. hep. = 23 / 1 a. coel. = 16 / 1	9,0

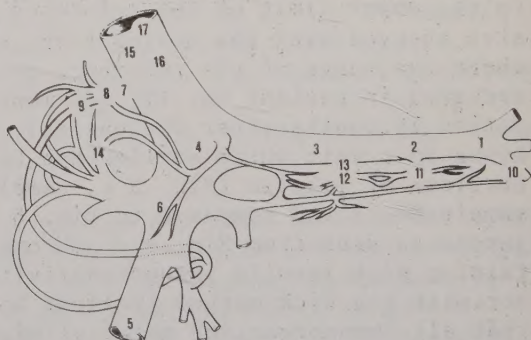


Fig. 3 a

Results of insulin determination with two immunoassay techniques (Wick/Heding) in patient No. III before operation.

	IRI (μ U/ml)		Glucose (mmol/l)
1. v. lienal.	= 72 / 99	v. hep. = 8 / 13	5,9
2. v. pancreat.	= 210 / 216	a. coel. = 6 / 0	
3. v. port. confl.	= 10 / 8	v. hep. = 58 / 11	
4.a. v. port. centr.	= 17 / 19	a. coel. = 2 / 1	5,0
b. v. port. centr.	= 15 / 19	v. hep. = 6 / 5	
c. v. port. centr.	= 13 / 23	a. coel. = 3 / 0	
5. v. mes. sup.	= 4 / 4	v. hep. = 6 / 9	5,2
6. GCT	= 132 / 104	a. coel. = 3 / 11	
7. GCT	= 165 / 104	v. hep. = 12 / 16	
8. v. mes. sup.	= 38 / 36	a. coel. = 2 / 1	5,0
9. v. PSPD	= 94 / 32	v. hep. = 14 / 16	
10. v. PSPD	= 54 / 84	a. coel. = 7 / 6	
11. v. port. centr.	= 13 / 11	v. hep. = 8 / 13	5,2
		a. coel. = 2 / 0	
		v. hep. = 2 / 9	
		a. coel. = 7 / 5	



Fig.3 b

Results of insulin determination with two immunoassay techniques (Wick/Heding) in patient No. III two months after operation.

	IRI (μ U/ml)		Glucose (mmol/l)
1. v. mes. sup.	= 8 / 1	v. hep. = 13 / 17	1,5
2. v. port. confl.	= 78 / 141	a. coel. = 10 / 19	
3. v. port. centr.	= 30 / 77	v. hep. = 11 / 20	
4. v. pancreat.	= 220 / 736	a. coel. = 9 / 17	1,5
5. v. pancreat.	= 208 / 652	v. hep. = 7 / 11	
6. v. lienal.	= 11 / 25	a. coel. = 7 / 13	
7. v. lienal.	= 152 / 368	v. hep. = 9 / 20	1,7
8. v. PSPD	= 120 / 134	a. coel. = 8 / 20	
9. v. PSPD	= 116 / 264	v. hep. = 7 / 11	
10. v. PSPD	= 112 / 248	a. coel. = 6 / 15	1,4
11. v. port. centr.	= 16 / 49	v. hep. = 7 / 11	
12. v. ASPD	= 6 / 15	a. coel. = 6 / 15	
13. v. ASPD	= 5 / 8		1,4
14. v. ASPD	= 4 / 0		
15. GCT	= 12 / 30		
16. GCT	= 14 / 40		1,4
17. GCT	= 5 / 17		
18. GCT	= 4 / 11	v. hep. = 5 / 14	
19. PSPD	= 100 / 272	a. coel. = 6 / 12	

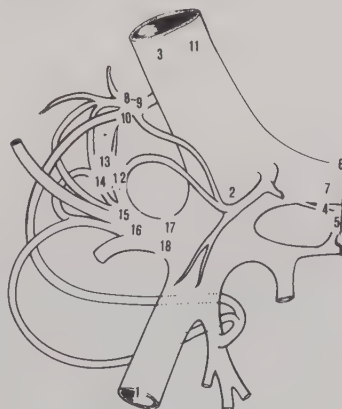


Fig.3 c

Results of insulin determination with two immunoassay techniques (Wick/Heding) in patient No. III twelve months after operation.

The tumor from patient No. II contained numerous insulin immunoreactive cells whereas glucagon, gastrin and HPP cells were absent. The tumor cells displayed only weak immunoreactivity when fixed with the formaldehyde-Bouin sequence, which we in numerous other cases have found to be the fixation of choice for demonstrating insulin immunoreactivity (15, 16). However, with diethylpyrocarbonate fixation, a procedure which in our hands gives slightly inferior results, intense immunoreactivity was seen in most tumor cells. In some sections apparently normal pancreatic tissue was detected outside the tumor. The extra-tumoral islets were of normal frequency and contained insulin, glucagon and HPP cells in normal proportions. In contrast to the tumor, the insulin cells reacted normally to different fixatives, i.e. they displayed intense insulin immunoreactivity upon formaldehyde-Bouin fixation and were slightly less immunoreactive when fixed with DEPC.

In the main portion of the resected specimens from patients Nos. III and IV the islet frequency was much increased. In all islets insulin, glucagon and HPP cells were found in normal proportions. In addition, quite a few insulin cells were found scattered in the exocrine parenchyma where they appeared to be squeezed in between neighbouring acinar cells. Some insulin cells were also found in the epithelium of small and medium sized ducts. No gastrin immunoreactive cells could be demonstrated. At the margins of the resected specimen the frequency of islets and of extrainsular cells decreased in both patients. By counting the number of islets under lower power magnification a semiquantitative estimate revealed that the islet frequency in the middle of the resected specimen exceeded the frequency of islets at the margins by a factor of 3 to 4. Despite intense search no tumor or adenoma-like structures were found and no signs of active or chronic inflammation were noted.

Discussion

In five patients with hyperinsulinism two tumors were localized pre-operatively by conventional localization procedures. Pancreatic vein catheterization revealed localized, pathologically high insulin concentrations in all five patients. The cause of the hyperinsulinemia was in 3 patients found to be a pancreatic tumor and in the remaining two a localized islet cell hyperplasia.

Pancreatic resections were easily performed according to the catheterization results concerning the venous anatomy and hormone differences. However, in patient No. II one of the two assays used failed to detect the tumor insulin. Also immunocytochemistry indicated that the insulin present in these tumor cells of this case reacted differently from insulin of normal beta-cells and of other insulinoma tissues. Conceivably, this abnormal immunoreactivity reflects a change in the amino acid sequence and/or the conformation of the tumor insulin (4). Thus, some insulinoma patients may have greatly elevated levels of bioactive hormone that are not detectable with all insulin radioimmunoassays.

	IRI (μ U/ml)		Glucose (mmol/l)
1. v. lienal.	= 11 / 17	v. hep. = 14 / 16 a. coel. = 12 / 14	4,0
2. v. lienal.	= 21 / 24		
3. v. lienal.	= 10 / 15		
4. v. pancreat.	= 245 / -		
5. v. pancreat.	= 245 / 270	v. hep. = 24 / 27 a. coel. = 21 / 25	
6. v. lienal.	= 14 / 20	v. hep. = 17 / 21 a. coel. = 15 / 18	
7. v. DP	= 15 / 13		
8. v. DP	= 13 / 19		
9. v. DP	= 25 / 30	v. hep. = 29 / 25 a. coel. = 17 / 23	4,8
10. v. portae	= 68 / 79		
11. GCT	= 72 / 71		
12. GCT	= 35 / 47		
13. GCT	= 63 / 61	v. hep. = 21 / 34 a. coel. = 17 / 26	
14. v. ASPD	= 235 / -		
15. v. mes. sup.	= 20 / 28		
16. v. mes. sup.	= 18 / 26		
17. v. port.	= 38 / 52		
18. v. port.	= 34 / 52		
19. v. port.	= 27 / -		
20. v. port.	= 54 / 65	v. hep. = 28 / 45 a. coel. = 16 / 20	5,0

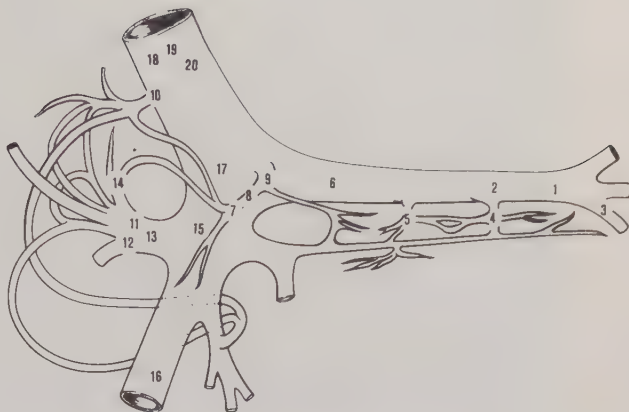


Fig. 4 a

Results of insulin determination with two immunoassay techniques (Wick/Heding) in patient No. IV before operation.

	IRI (μ U/ml)		Glucose (mmol/l)
1. v. lienal.	= 23	v. hep. = 5 a. coel. = 4	5,4
2. GCT	= 22	v. hep. = 5 a. coel. = 2	
3. v. DP	= 12	v. hep. = 5 a. coel. = 2	
4. v. DP	= 22		4,9
5. v. portae	= 24		
6. v. mes. sup.	= 5		
7. v. mes. sup.	= 2		
8. v. portae	= 1		
9. v. portae	= 10	v. hep. = 5 a. coel. = 4	4,9



Fig. 4 b

Results of insulin determination with the Wick technique in patient No. IV two months after operation.

	(RI μU/ml)		Glucose (mmol/l)
1. v. port. centr.	= 29/19	v. hep. = 19/5	5,2
2. v. col.	= 18/13	a. coel. = 20/13	
3. v. DP	= 25/14		
4. v. DP	= 24/19		
5. v. ASPD	= 24/34		5,3
6. v. ileum.	= 15/10	v. hep. = 33/28	4,5
7. v. GCT	= 19/15	a. coel. = 28/30	
8. v. GCT	= 19/19		
9. v. ASPD	= 13/17		3,3
10. v. ilenal. dist.	= 14/17		
11. v. ilenal.	= 16/18		
12. v. ilenal.	= 25/27		
13. v. pancreat.	= 655/560		
14. v. pancreat.	= 6200/5200	a. coel. = missed	3,5
15. v. ilenal.	= 21/26	v. hep. dx. = 55/41	
16. v. ilenal.	= 179/308		
17. v. ilenal.	= 86/116		3,7
18.-22. v. PSPD	= 10/4 13/17 a. coel. = 14/5 13/8 11/13 v. hep. = 35/46 12/15		3,6

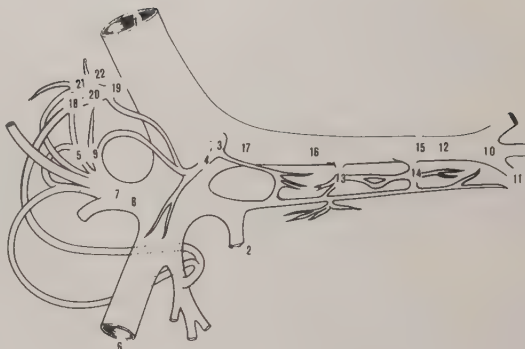


Fig. 5

Results of insulin determination with two immunoassay techniques (Wick/Heding) in patient No. V before operation.



Fig. 6 a

Pancreatic phlebography in patient No. III (fig. 3 a, sample no 12-1



Fig. 6 b

Pancreatic phlebography in patient No. III (fig. 3 b, sample no. 2)



Fig. 6 c

Pancreatic phlebography in patient No. III (fig. 3 c, sample no. 4-5).

Proinsulin - and C-peptide determinations have been reported to be useful for the diagnosis of insulin secreting tumors (1, 10, 21). In patients Nos. II, III and IV both peripheral serum and serum withdrawn from tumor veins contained normal ratios of proinsulin:insulin.

During this study only one complication was noted. This occurred in patient No. II in whom the preoperative catheterization caused a hepatic hematoma (2-3 cm in diameter). The hematoma has, however, not troubled the patient.

Conclusions

Simultaneous aortal, caval and pancreatic vein catheterization with blood sampling for hormone assay seem to be very useful for localizing insulinomas and islet cell hyperplasias. Knowledge of the pancreatic venous anatomy and the arteriovenous insulin difference guides the surgeon to correct resections of even small tumors or hyperplasias.

However, the accuracy of this method rests with the sensitivity and specificity of the radioimmunoassay technique employed. Hence tumor insulin may be detectable with one type of antibody but undetectable with another. The extent to which such abnormal tumor insulins are produced is currently unknown. The tripple catheterization technique seems to be a useful localization procedure also for recurrences. The method is advocated for in all patients with proven or suspected gastrointestinal endocrine tumors.

	Fig. no	Sample no	Proins.	Ins.	IRI (μ U/ml)
<u>Pat. no I postop.</u>	1 b.				
v. ASPD		1	0.15	0.85	86
v. DP		7	0.08	0.92	134
<u>Pat. no II preop.</u>	2 a.				
v. pancreat.		22	0.05	0.95	35
<u>Pat. no III preop.</u>	3 a.				
v. pancreat.		11	0.01	0.99	950
v. pancreat.		12	0.02	0.98	1130
v. hep.		12	0.05	0.95	19
a. coel.		12	0.15	0.85	17
<u>Pat. no III postop.</u>	3 b.				
v. pancreat.		2	0.05	0.95	210
v. hep.		2	0.25	0.75	58
<u>Pat. no IV preop.</u>	4 a.				
v. lienal.		4	0.12	0.88	245
v. ASPD		14	0.07	0.93	235
a. coel.		5	0.15	0.85	21
<u>Pat. no IV postop.</u>	4 b.				
v. pancreat.		1	0.02	0.98	23
v. DP		4	0.06	0.94	22
<u>Y.C. ref. patient</u>					
v. DP			0.14	0.86	34

Table II

Fraction of total insulin immunoreactivity (IRI) composed by pro-insulin like components (PLC) and insulin.

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